

FIG.1

MOLECULES INVOLVING, etc. REPLACEMENT SHEET 2/7 UPPER PRIMER 1044 SEQ ID NO:1 LOWER PRIMER LABEL AMPLIFICATION OF TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dUTP, AND dCTP AND  $^{32}\text{P}$  LABELLED LOWER PRIMER. 952 U U U U U U 3' SEQ ID NO:2

U U U 5' SEQ ID NO:3 - Treatment with exonuclease I AND SHRIMP ALKALINE PHOSPHATASE. - TREATMENT WITH UDG. -TREATMENT WITH NaOH AT 95°C 1020 1044 (ONLY LABELLED FRAGMENTS SHOWN HERE) SEQ ID NO:4 REMOVAL OF 3' PHOSPHATE BY TREATMENT WITH T4 PNK SEQ ID NO:5 (ONLY LABELLED FRAGMENTS SHOWN HERE) LINEAR AMPLIFICATION OF DNA (952 TO 1044) USING LABELLED UPSTREAM FRAGMENT FOLLOWED BY ANALYSIS ON DENATURING POLYACRYLAMIDE GEL, FOLLOWED BY AUTORADIOGRAPHY. 952 1044 93MER, EXTENDED UPSTREAM FRAGMENT SEQ ID NO:6 952 = SEQ ID NO:1 1044

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FIG.2

TEMPLATE DNA

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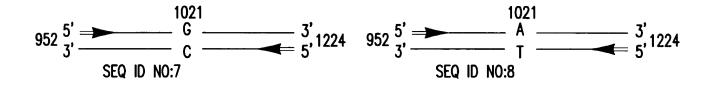
MOLECULES INVOLVING, etc. REPLACEMENT SHEET

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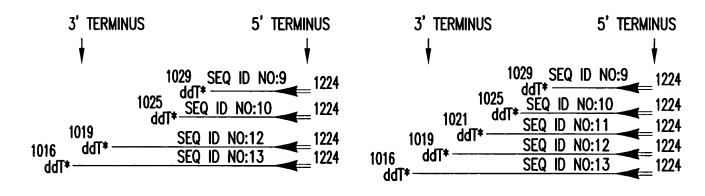
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- AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, datp, dctp and 1/20 ratio of dutp to dttp.



- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
- TREATMENT WITH UDG.
- TREATMENT WITH NaOH AT 95°C
- DNA IS PRECIPITATED.
- TREATMENT WITH T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF <sup>33</sup>P-LABELLED ddttp\* and unlabelled ddgtp, ddatp, and ddctp.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.

DETECTION OF EXTENDED LABELLED FRAGMENTS BY PAGE AND AUTORADIOGRAPHY

<del></del>	

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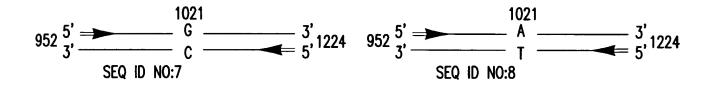
MOLECULES INVOLVING, etc.

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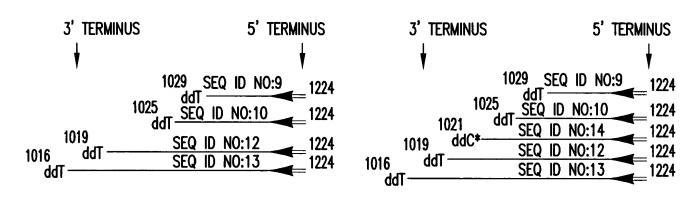
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- AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dCTP AND 1/20 RATIO OF dUTP TO dTTP.



- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
- Treatment with UDG.
- TREATMENT WITH NaOH AT 95°C
- DNA IS PRECIPITATED.
- TREATMENT WITH T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF 33P-LABELLED ddctp\* and unlabelled ddGTP, ddATP, AND ddTTP.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.

1021 **SEQ ID NO:14** ddC\*

DETECTION OF EXTENDED LABELLED FRAGMENTS BY PAGE AND AUTORADIOGRAPHY

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6390 UPPER PRIMER A SEQ ID NO:15 6443

5' AACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3'
3' TTGAACACCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

T LOWER PRIMER

AMPLIFICATION OF TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, datp, dutp, and dctp.

SEQ ID NO:16

AMPLIFIED MUTANT ALLELE

- 5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' SEQ ID NO:17

**SEQ ID NO:18** 

AMPLIFIED MUTANT ALLELE

- 5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' SEQ ID NO:19
  - TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATASE.
  - TREATMENT WITH UDG.
  - TREATMENT WITH ENDO IV.

SEQ ID NO:20 NORMAL UPSTREAM FRAGMENT
3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

SEQ ID NO:21 MUTANT UPSTREAM FRAGMENT
3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

6/7 REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.1 SEQ ID NO:22 5' GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3' X X3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' NORMAL UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF  $\alpha^{\ensuremath{32}}\mbox{PdCTP}$ **SEQ ID NO:20** FOLLOWED BY DENATURING PAGE **SEQ ID NO:24** 3' CGACATTTGCTGCCGGTCAAAGTACGTCCCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' \* \*\* \* \*\* В REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.2 SEQ ID NO:25 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACCGC 3' XXXXX 3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' NORMAL UPSTREAM FRAGMENT **SEQ ID NO:20** PCR AMPLIFICATION IN PRESENCE OF  $\alpha^{32}$ PdCTP FOLLOWED BY DENATURING PAGE X C REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.2 SEQ ID NO:25 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3' 3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' MUTANT UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF  $\alpha^{32}$ PdCTP SEQ ID NO:21 FOLLOWED BY DENATURING PAGE **SEQ ID N0:26** 3' CGACATTTGCTGCCGGTCAAAGTACGTCCTAGGTACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' 66MER D REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.1 SEQ ID NO:22 5' GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3' 3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' MUTANT UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF  $\alpha^{\ensuremath{32}\xspace}\text{PdCTP}$ **SEQ ID NO:21** FOLLOWED BY DENATURING PAGE MISMATCHES DENOTED BY X X  $^{32}$ P Label denoted by \*

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REPLACEMENT SHEET

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MOLECULES INVOLVING, etc. REPLACEMENT SHEET **SEQ ID NO:27** Α TEMPLATE OLIGO 1 GGTAGTTGGAGCTGGTGGCG **SEQ ID NO:20** CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG CCATCAACCT\* 3' REPORTER 5' NORMAL UPSTREAM FRAGMENT 0LIG0 1 LIGATION REACTION FOLLOWED BY **SEQ ID NO:28** DENATURING PAGE **SEQ ID NO:29** CCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' 47MER **SEQ ID NO:27** TEMPLATE OLIGO 1 **GGTAGTTGGAGCTGGTGGCG SEQ ID NO:20** AACCTCGACC\* ĊĠĂĊĊĂĊĊĠĊATCCGTTCTCACGGAACTGCTATGTCG 3'RFPORTER 5' NORMAL UPSTREAM FRAGMENT OLIGO 2 LIGATION REACTION FOLLOWED BY SEQ ID NO:30 DENATURING PAGE AACCTCGACC\* SEQ ID NO:30 10MER **SEQ ID NO:31** C TEMPLATE OLIGO 2 TTGGAGCTGGTGGCGTAGGC **SEQ ID NO:21** .////////// AACCTCGACC\* ACCGCATCCGTTCTCACGGAACTGCTATGTCG MUTANT UPSTREAM FRAGMENT 3 REPORTER 5 0LIG0 2 LIGATION REACTION FOLLOWED BY SEQ ID NO:30 DENATURING PAGE SEQ ID NO:32 AACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' 42MER **SEQ ID NO:31** D TEMPLATE OLIGO 2 TTGGAGCTGGTGGCGTAGGC SEQ ID NO:21 CCATCAACCT\* ACCGCATCCGTTCTCACGGAACTGCTATGTCG 3' REPORTER 5' MUTANT UPSTREAM FRAGMENT OLIGO 1 32P LABEL DENOTED BY \* LIGATION REACTION FOLLOWED BY SEQ ID NO:28 DENATURING PAGE ✓ DENOTES BASEPAIRING SEQ ID NO:28 CCATCAACCT\* FIG.7

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3 '

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10MER

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